Gastric Emptying and Intestinal Absorption of Ingested Water and Saline by Hypovolemic Rats

by

Michael R. Bykowski

Bachelor of Philosophy, University of Pittsburgh, 2006

Submitted to the Graduate Faculty of

School of Arts & Sciences in partial fulfillment

of the requirements for the degree of

Master of Science

University of Pittsburgh

2007

UNIVERSITY OF PITTSBURGH

SCHOOL OF ARTS & SCIENCES

This thesis was presented

by

Michael R. Bykowski

It was defended on

August 6, 2007

and approved by

Edward Stricker, University Professor, Department of Neuroscience

Alan Sved, Professor, Department of Neuroscience

Linda Rinaman, Associate Professor, Department of Neuroscience

Thesis Director: Edward Stricker, University Professor, Department of Neuroscience

Gastric Emptying and Intestinal Absorption of Ingested Water and Saline by Hypovolemic Rats

Michael R. Bykowski

University of Pittsburgh, 2007

Detection of blood volume deficits alters a rat's motivational state by stimulating thirst and salt appetite. In consequence, PEG-treated rats with established hypovolemia adaptively drink both water and hypertonic NaCl solution; indeed, they quickly alternate between drinking both fluids and concoct a mixture isotonic to body fluids – a concentration that is most effective in repairing plasma volume deficits without perturbing pOsm. However, their plasma volume deficits cannot be restored until ingested fluid is absorbed from the GI tract. The present experiment sought to address the issue of whether ingested water accelerates ingestion, gastric emptying, and small intestinal absorption of 0.30 M NaCl. In fact, ingestion of both water and 0.30 M NaCl did accelerate fluid delivery into the systemic circulation. Moreover, as a consequence of fluid leaving the GI tract more quickly, GI distension signals associated with inhibition of fluid intake are quickly removed, leading to larger fluid intakes. The unique behavior of PEG-treated rats corresponds to restoration of their body fluid deficits, including behavioral, physiological, and hormonal aspects of body fluid homeostasis. Clearly, co-existence of thirst and salt appetite is an adaptive behavioral response to hypovolemia.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1.0 INTRODUCTION

Maintenance of blood volume and body fluid concentration is crucial for an animal's survival. Even minor perturbations in plasma osmolality (pOsm) can affect the rate of biochemical reactions. Accordingly, body fluid homeostasis is tightly regulated through multiple behavioral and physiological processes. For example, hypovolemia elicits a variety of neural and hormonal *input* signals that elicit a variety of neural and hormonal *output* signals, which collectively have the net effect of enhancing water and salt retention in urine. Adaptively, the physiological deficits also alter a rat's motivational drive state to increase water and salt intake.

The hypothalamus regulates the pituitary gland and is generally regarded as an integration site of autonomic function; it is the apex of the hypothalamic-pituitary-adrenal axis. Neurons within the hypothalamus receive inputs from the organum vasculosum of the lamina terminalis (OVLT) and other circumventricular organs, which detect perturbations in systemic pOsm (and plasma Na^+ concentration) through osmosensitive (and Na^+-sensitive) cells. Elevated pOsm stimulates neurons within the paraventricular (PVN) and supraoptic nuclei (SON) of the hypothalamus to secrete vasopressin (VP) and oxytocin directly into the blood circulation via the hypophyseal vein. It is well-established that VP and oxytocin have antidiuretic and natriuretic effects, respectively. In parallel to these physiological responses, signals emanate from the median preoptic nucleus, subfornical organ, and OVLT to stimulate drinking.

The volume within the cardiovascular system is another component of body fluid homeostasis. A reduction in blood volume compromises delivery of nutrients and other blood-borne molecules to body tissues as well as removal of waste products. Subcutaneous injection of

polyethylene glycol (PEG) solution in rats sequesters extracellular fluid in the interstitium local to the injection site, resulting in depletion of plasma volume. Rats detect blood volume depletion through mechanosensitive receptors situated on the outer membranes of the great veins and right atrium. These receptors then transduce mechanical deformation into a bioelectric signal, which is sent to the nucleus of the solitary tract via the glossopharyngeal and vagus nerves and their branches. Additionally, renal baroreceptors elicit a compensatory response by activation of the renin-angiotensin system. Baroreceptor neural signals as well as blood-borne signals are responsible for mediating VP secretion and the autonomic responses to hypovolemia. A drop in renal perfusion pressure decreases glomerular filtration rate, and thus limits urinary output – all of which effectively reduce aggravation of blood volume deficits.

In addition to circulatory and renal compensatory mechanisms, behavioral mechanisms support restoration of blood volume deficits. Detection of blood volume deficits results in the generation of neural and hormonal signals, which are translated into water- and salt-seeking behaviors. That is, hypovolemic rats develop thirst and salt appetite. When given access to water and 0.50 M NaCl, PEG-treated hypovolemic rats adaptively drink both fluids and concoct a solution that is isotonic to their body fluids to compensate for intravascular fluid deficits (Stricker, 1981; Stricker & Jalowiec, 1970). This isotonic mixture is the most effective concentration in repairing their body fluid deficits while preventing intracellular dehydration and osmotic dilution. However, rats made hypovolemic by PEG treatment stop their initial drinking bout of water and saline well before plasma volume deficits are repaired (Smith et al., 2007). Because essentially all of the ingested fluid remains in the gastrointestinal (GI) tract by the time the initial bout ended, it has been hypothesized that a pre-systemic inhibitory signal inhibits fluid

consumption (Smith et al., 2007), presumably mediated through the NTS and area postrema (Stricker et al., 1997; Curtis et al., 1996).

Thrasher et al. (1981) have observed a pre-systemic signal (i.e., the act of drinking) that inhibits thirst and VP secretion in water-deprived dogs before systemic blood was affected. Furthermore, thirst and VP secretion were rapidly inhibited regardless of the concentration of the ingested fluid and whether or not it emptied from the stomach. However, inhibition of thirst by oropharyngeal signals is not present in rats; VP secretion and thirst are not inhibited after ingestion of isotonic saline or when rats drink with open gastric fistulae (Davis et al., 2002; Huang et al., 2000; Waldbillig & Lynch, 1979). Accumulating evidence suggests that the pre-systemic inhibitory signal stems from distension of the stomach and small intestine (Bykowski et al., in press, Hoffmann et al., 2006, Smith et al., 2007, Stricker et al., 2007).

Similarly, gastric emptying seems to be influenced by pre-systemic factors and to vary in response to the content of the material being emptied. For example, emptying of NaCl solution slows in proportion to its hypertonicity. It has been hypothesized that the concentration of the emptied fluid is detected by visceral osmoreceptors (Choi-Kwon et al., 1990). These receptors have been proposed to be situated near the proximal portion of the small intestine, a location that allows "sampling" of ingested fluid before it reaches the systemic blood circulation. Detection of hypertonic NaCl solution by these receptors has been observed to significantly decrease gastric emptying, increase water intake, and increase VP secretion before an increase in systemic pOsm has occurred (which has traditionally been thought to be the stimulus for VP secretion after salt ingestion) (Bykowski et al., in press; Smith et al., 2007; Stricker et al., 2007; Manesh et al., 2006; Hoffmann et al., 2006; Stricker & Hoffmann, 2005; Stricker et al., 2002).

Concurrent water and hypertonic saline intake is advantageous to the hypovolemic rat because both water and NaCl are needed to repair plasma volume deficits. However, it is clear that the behavior of transporting fluid from the external environment into the stomach is not enough for the hypovolemic animal's survival even if it is the ideal fluid for restoring plasma volume deficits; the nervous and endocrine systems must act together with the GI tract to deliver the fluid into the systemic blood circulation. More specifically, the ingested fluid actually is not effective in restoring blood volume deficits until it empties from the stomach and is absorbed into the circulation. The rate at which fluid moves through the GI tract into the systemic circulation is governed primarily by the rate of gastric emptying; in fact, gastric emptying appears to be the rate-limiting step.

The goals of the present experiments were to examine the effects of PEG-induced hypovolemia on the consumption, gastric emptying, and intestinal absorption of water and 0.30 M NaCl during the initial drinking episode in a two-bottle, 16-h delayed access test. Since gastric emptying of hypertonic NaCl solutions in rats has been shown to be much slower than emptying of isotonic saline (Bykowski et al., in press; Smith et al., 2007; Stricker et al., 2007), these experiments test the hypothesis that concurrent water consumption accelerates movement of ingested 0.30 M NaCl through the GI tract and into the systemic blood circulation.

2.0 METHODS

Animals. Adult male Sprague-Dawley rats (Harlan Laboratories) weighing 325-425 g on the day of the terminal experiments were housed singly in wire-mesh cages in the Department of Neuroscience vivarium. The colony rooms were maintained at constant temperature (22-23 ºC) and with a fixed light-dark cycle (lights on from 7 a.m. to 7 p.m.). All rats were given >1 wk of *ad libitum* access to pelleted laboratory chow (5001, Purina) and tap water before the experiment.

Experimental protocols. Experimental protocols were reviewed by and received approval from the Institutional Animal Care and Use Committee of the University of Pittsburgh.

Experiment 1. The goal of this experiment was to investigate the transport of water and hypertonic saline – when ingested in close temporal proximity – through the GI tract. More specifically, we wondered whether concurrent ingestion of water and hypertonic saline would increase gastric emptying and absorption of ingested fluid from the small intestine relative to when rats drink hypertonic saline alone.

During the four days preceding the experiment, all rats had *ad libitum* access to 0.30 M NaCl in addition to water and food, in order to give them experience in drinking the hypertonic solution. Rats were given interscapular, subcutaneous injections of 5 ml of 30% PEG solution (w/w), after which they were deprived of food, water, and saline for 16 h. Subsequently, these rats were given access to water and 0.30 M NaCl in a two-bottle drinking test, and then they

were separated into two groups based on which solution they consumed first: "water-first" (n = 7) or "saline-first" (n = 13). Additional animals (n = 10) were maintained for 4 days on sodium-deficient (NaD) diet before injection of 5 ml of 20% PEG, which was used instead of the 30% PEG solution to limit the degree of hypovolemia. (In explanation, we feared that severe colloid-induced hypovolemia would debilitate sodium-deprived rats and might even kill them.) We assumed that dietary NaCl deprivation would encourage PEG-treated animals to drink saline before consuming water, as observed previously (Stricker, 1981). The focus of the experiment was the initial drinking episode after access to fluid was restored 16 h after PEG injection. Animals were sacrificed before, during, or immediately after this episode.

A few drops of green food dye (McCormick & Co., Hunt Valley, MO) were added to the drinking fluids, which allowed us to track the ingested fluid within the GI tract. During the drinking tests, fluids were presented in graduated burettes and saline and water intakes were recorded (± 0.1 ml) each time the rat switched from one solution to the other. Some rats (water-first, n = 3; saline-first, n = 2) were interrupted from drinking by the experimenter in order to obtain information about the distribution of the ingested fluid in the GI tract before the end of the episode. All other rats were killed once they accrued 2 min of continuous non-drinking activity, which defined the end of the first drinking episode (Smith et al., 2007). Within 10 s after the test ended, all rats were decapitated, trunk blood was collected, and the stomachs and segments of small intestine that contained dye were removed for analysis (see below). We assumed from the absence of green dye that a measurable amount of residual ingested fluid was not present in the rat's mouth and esophagus.

Analysis of Tissues. After decapitation, trunk blood was collected in chilled heparinized collection tubes (143 USP sodium heparin; Becton Dickinson, Franklin Lakes, NJ), which were

placed in ice until they were centrifuged (10,000 x *g* for 5 min at 4°C). The plasma supernatant was collected and plasma Na^+ (pNa) was analyzed later using a sodium-sensitive electrode (±1 mEq/L; Beckman Coulter, Synchron EL-*ISE* model 4410, Brea, CA) while plasma protein concentration was measured (±0.1 g/dl) using a refractometer. Immediately after decapitation, the visceral organs were exposed by a midline incision on the abdomen. Hemostats were clamped at the junction of the pylorus and small intestine, the junction of the esophagus and stomach, and at the farthest point to which the green dye had traveled into the small intestine, in that order. (The third hemostat was clamped at the junction of the small intestine and cecum on the one occasion when green dye traveled into the cecum.) Subsequently, the stomach and the portion of small intestine containing dyed fluid, stripped of adhering blood vessels and connective tissues, were removed and placed into separate beakers, which were covered with Parafilm to prevent evaporation. The stomach was opened and gastric contents were removed and weighed. The length of the small intestinal segment removed was measured (±1 cm) and weighed (±1 mg). The stomach contents and intestinal segment were desiccated until constant weight (1-2 days at 60 ºC).

To summarize, our goal was to analyze in each of 30 PEG-treated rats all blood samples for plasma concentrations of Na^+ and protein, gastric chyme and small intestine (separately) for dry matter and water, and intestinal distance traversed by the ingested fluid. These analyses sum to a total of 210 individual measurements, of which 12 (6%) were lost due to procedural errors.

For purposes of comparison, data from C.A. Smith's master's thesis and the recently published article based on it (Smith et al., 2007) are included in certain figures below. The methods used in her thesis were identical to those employed in the present experiment except

that during the previous drinking test rats had access to only one bottle containing either water, 0.15 M NaCl, or 0.30 M NaCl.

Calculations. In order to distinguish between the ingested fluid and gastric fluid that was present before testing, we corrected for fluid associated with gastric chyme. This volume was calculated by Smith et al. (2007) using PEG-treated rats (n = 7) that were injected with 5 ml of 30% PEG at 5 p.m., deprived for 16 h of food, water, and saline, and decapitated at 9 a.m. the following morning without having been tested. Trunk blood, stomach, and small intestine were collected from each animal and treated as described above. A scatter plot was constructed expressing stomach liquids of individual animals (in ml, on the y-axis) as a function of stomach solids (in g, on the x-axis). The resulting trendline, $y = 2.0475x + 0.1331$, was used to correct for the volume of fluid associated with gastric solids in each rat tested. This equation is similar to others that were obtained previously in this laboratory. These corrections assume that the amount of food present in the stomach, as well as fluid associated with food, remained constant throughout the testing period.

Another correction was made in order to discriminate between orogastric secretions and ingested fluid. Briefly, in previous studies (Stricker et al., 2007; Stricker & Hoffmann, 2005) DOCA-treated rats and water-deprived rats were fitted with gastric fistulae and adapted to cages that allowed drainage and collection of gastric fluid. On average, 12.6% more fluid drained from the rats' stomachs than was consumed. For this reason, we estimated the volume of orogastric secretions as 12.6% of the volume of fluid consumed by PEG-treated rats.

The concentration of stomach contents was determined using the following steps. The stomach contents were desiccated for 1-2 days to evaporate the water associated with the stomach contents. Next, a measured volume of water was added to the individual beakers

containing dried stomach contents, which was then mixed thoroughly. This mixture was covered with Parafilm to prevent evaporation and stirred periodically for 15 min, after which it was centrifuged (10,000 x *g* for 2 min) and the sodium concentration of the supernatant fluid was analyzed using a sodium-sensitive electrode. The sodium concentration of this sample was multiplied by the measured volume initially added to the stomach contents, and that product gave the number of milliequivalents of sodium in the sample. Finally, the number of milliequivalents of sodium was divided by the volume of stomach liquids, resulting in the Na^+ concentration of stomach contents.

Another correction was made to distinguish between fluid associated with the small intestinal lining and fluid within the intestinal lumen. Various lengths of small intestine were collected from 7 control rats injected sc with 30% PEG and deprived of food, water, and saline for 16 h (Smith et al., 2007), as described above, and then were sacrificed without being given access to drinking fluid. A scatter plot was constructed expressing the total small intestinal volume of individual animals (in ml, on the *y*-axis) as a function of intestinal segment length (in cm, on the *x*-axis). The associated trendline, $y = 0.038x - 0.1202$, was used to correct for fluid associated with intestinal tissues per cm. This equation is similar to others that were obtained in this laboratory previously. The weight of the dry lining of small intestine was considered to be negligible. The value calculated from the trendline was multiplied by the length of small intestinal segment collected from each animal tested, and that product was subtracted from the total water volume of the small intestinal segment. The resulting value was considered to be the volume of fluid in the intestinal lumen.

In order to calculate the percent of ingested fluid that emptied from the stomach, the fluid remaining in the stomach (corrected for fluid associated with gastric chyme) was subtracted from

the volume of intake (corrected for orogastric secretions) and then divided by the volume of intake (corrected for orogastric secretions) and multiplied by 100. (Calculations that involved intestinal lumen volume were not done in the one rat in which the dyed ingested fluid was visible in the cecum.)

The percent of fluid emptied from the stomach that was absorbed from the small intestine was calculated by dividing the difference between the volume of fluid emptied from the stomach and the volume in the small intestinal lumen by the volume of fluid emptied from the stomach, all multiplied by 100.

Statistical Analyses. All data are presented in scatterplots or as means ± SE. Statistical reliability of observed differences was determined using a one-way ANOVA and *t*-tests, except in Fig. 6 (below) where a chi-square analysis was employed. Regression equations were calculated by the method of least squares and significance was determined using Pearson's correlation coefficients. Differences in the slopes of lines were determined by converting individual data points into ratios of the two variables (i.e., *y*-value/*x*-value) and then comparing the groups by ANOVA. $P < 0.05$ was considered to be statistically significant.

3.0 RESULTS

In a two-bottle drinking test, PEG-treated rats drank water and 0.30 M NaCl alternately and in small bouts. Fig. 1 shows the initial drinking episode of a representative animal, which had 9 drinking bouts. That was an unusually large number of bouts. As shown in Table 1, rats that drank water first had fewer bouts per episode than rats that consumed 0.30 M NaCl first or rats that had been maintained on NaD diet (both *P*s < 0.05). The average time of each bout, total time spent drinking, and total volumes consumed were comparable for all rats that drank without interruption in a two-bottle test.

Fig. 2A presents the drinking bouts of individual rats that drank saline first, plotted so as to emphasize the relative amounts of saline and water that were consumed in the first drinking episode; data from the animal shown in Fig. 1 is highlighted by a solid gray line. Figs. 2B and 2C present comparable data from rats that drank water first and from rats in the NaD diet group, respectively. The diagonal lines in each figure indicate consumption of equal amounts of water and 0.30 M NaCl (i.e., intake of 0.15 M NaCl). The proximity of the individual symbols to the diagonal line indicates that more than half the rats (i.e., 17 of 30) consumed a mixture that was not too different from being isotonic to body fluids (i.e., 0.11 M – 0.18 M NaCl). However, 7 rats drank much more of one fluid than the other. It is noteworthy that the 4 rats (encircled symbols in Figs. 2A and 2C) that consumed 0.30 M NaCl almost exclusively differed from the other rats in several other ways, as will be mentioned below, whereas the 3 rats that consumed

mostly water were generally similar in other ways to rats that consumed a nearly isotonic mixture.

Rats consumed water and saline at a similar rate (1.1 ± 0.04 ml/min) during the first episode regardless of how much of the two fluids they consumed (Fig. 3). This rate was similar to that seen when PEG-treated rats drank water, 0.15 M NaCl, or 0.30 M NaCl in a one-bottle test (Smith et al., 2007). A continuous pause time of 2 min was used to define the end of the bout, but three saline-first rats and one NaD diet rat drank with relatively lengthy pauses that were not quite long enough to meet the criterion for terminating the drinking episodes, and consequently they drank at unusually slow rates for relatively long periods of time. Two of the four encircled symbols represent rats that consumed a hypertonic mixture in this manner.

As expected, PEG-treated rats were significantly more hypovolemic than non-injected control animals, as indicated by significant increases in plasma protein concentration. The plasma protein concentrations were comparable regardless of whether PEG-treated rats drank water first or saline first or if they had been maintained on NaD diet (8.3 ± 0.2, 8.4 ± 0.3, and 8.3 ± 0.3 g/dl, respectively; all *P*s <0.001 in comparison to untreated control values of 6.1 ± 0.1 g/dl but not in comparison to PEG-treated control values of 9.0 ± 0.5 g/dl). These values also resembled those reported in rats drinking 0.15 M NaCl in a one-bottle test (8.2 ± 0.4 g/dl; Smith et al., 2007).

As shown in Fig. 4, the rate at which fluid emptied from the stomach generally increased as the drinking rate increased ($r = 0.75$; $P < 0.001$). This relation was observed over a very wide range of drinking rates (~0.4 to ~1.5 ml/min). Fig. 5 shows the amount of ingested fluid that was emptied from the stomach as a function of fluid intake. In a previous study (Smith et al., 2007), rats emptied 0.15 M NaCl much faster than they emptied 0.30 M NaCl. In the

present experiment, when water was ingested after 0.30 M NaCl in the same drinking episode, this fluid mixture generally emptied faster than 0.30 M NaCl alone although the results did vary. The fluid mixture sometimes emptied like 0.15 M NaCl, sometimes like 0.30 M NaCl, and sometimes at a rate between the rates associated with those two concentrations. Similar results were observed in rats that drank water before 0.30 M NaCl and rats that had been maintained on NaD diet. Note that the animals that consumed the most concentrated mixture of fluids (i.e., the three encircled symbols in Figs. 4 and 5) emptied fluid at a rate similar to rats that drank 0.30 M NaCl alone. Fig. 6 shows that gastric emptying was proportional to the concentration of the fluid mixture that emptied from the stomach. That is, the most concentrated fluid emptied the least quickly (P <0.001), whereas the rats with stomach concentrations ranging from 28 - 154 mM NaCl emptied at rates that were similar to each other.

Absorption from the small intestine was also affected by consumption of water and 0.30 M NaCl when the two fluids were consumed in close temporal proximity. As shown in Fig. 7, rats that drank both water and 0.30 M NaCl, in whichever order, absorbed ingested fluid at the rate of ~0.25 ml/min (r = 0.82, P <0.001), which was similar to the absorption rates in rats that drank water or 0.15 M NaCl alone but were much larger than the rate of fluid absorption in rats that drank 0.30 M NaCl alone (Smith et al., 2007). However, there were four rats (encircled symbols in Fig. 7) that consumed both fluids but had little or no net absorption, like rats that consumed 0.30 M NaCl alone. Interestingly, these rats drank much more 0.30 M NaCl than water and thus consumed a mixed solution closer to 0.30 M NaCl than to 0.15 M NaCl (0.23 M, 0.25 M, 0.26 M, and 0.26 M NaCl).

4.0 DISCUSSION

The main goals of the present experiment were to determine the dynamic changes in body fluids that result from consumption of water and 0.30 M NaCl by PEG-treated rats in a two-bottle drinking test. More specifically, we investigated whether gastric emptying and absorption of 0.30 M NaCl were increased when hypovolemic rats consume this saline solution either just before or soon after drinking water. The results indicated that concurrent ingestion of water and 0.30 M NaCl facilitated movement of hypertonic solution through the GI tract into the general circulation. These results are discussed in the following three sub-sections: Drinking, Gastric Emptying, and Absorption from the Small Intestine.

4.1 DRINKING

When injected subcutaneously, PEG solution can produce a substantial decrease in blood volume (Stricker, 1966; Stricker, 1968). Extravascular PEG treatment creates an oncotic pressure that progressively sequesters isotonic, protein-free extracellular fluid at the injection site, slowly diminishing plasma volume. Because isotonic fluid is most effective in repairing body fluid volume deficits, it is appropriate that hypovolemia induces both thirst and salt appetite in rats. Accordingly, in the present experiments, PEG-treated rats drank both water and 0.30 M NaCl within the same drinking episode in an alternating fashion and, more interestingly, many

rats drank the solutions in approximately equal proportions and thereby concocted a mixture isotonic to body fluids (Figs. 1 and 2).

Previous studies have found that PEG-treated rats drank both water and hypertonic saline in two-bottle drinking tests, but they did so in a different drinking pattern than that observed in the present experiments. In 1992, Stricker et al. reported a detailed analysis of drinking behavior in PEG-treated rats over a 23-hr period. Hypovolemic rats were observed to drink water almost exclusively for the first several hours, but then they drank both water and hypertonic saline in such a way as to concoct a solution nearly isotonic to body fluids. These observations, in part, led to the hypothesis that thirst must first appear to encourage water intake, which dilutes body fluids and thereby blunts neurohypophyseal secretion of oxytocin (OT; Stricker & Verbalis, 1986). The concurrent release of OT as a neurotransmitter within the brain has been proposed to inhibit salt appetite in rats (Stricker & Verbalis, 1996); thus, the blunted secretion of OT in dilute PEG-treated rats has been proposed to allow for the expression of salt appetite in hypovolemic rats. Furthermore, oxytocinergic projections have been identified from the PVN in the hypothalamus to the medulla (Sawchenko & Swanson, 1982). Interestingly, activation of a subset of cells in the NTS has been shown recently in multiple models of salt appetite, including PEG-induced hypovolemia (Geerling et al., 2006; Geerling & Loewy, 2006), but not of thirst (Geerling & Loewy, 2007).

In contrast to expectations, rats in the present experiments demonstrated different ingestive behaviors. Immediately after being given access to the two fluids, all rats drank hypertonic saline in addition to water in the initial drinking episode, and most rats (19 of 30) drank hypertonic saline before consuming water even though pNa was not diluted (143 ± 0.3 meq/l) (Figs. 2A-C). Hypovolemia is a substantial stimulus for OT secretion (Stricker &

Verbalis, 1986) and no doubt plasma OT was elevated in the present experiments; nonetheless, rats evidently had a salt appetite. In light of previous observations (Stricker, 1981) that rats drink water almost exclusively for several hours before drinking 0.50 M NaCl, it was a surprise that rats drank hypertonic saline so early in the present two-bottle test. However, it must be emphasized that rats in the present experiments were given 0.30 M NaCl and water to drink 16 h after PEG injection, whereas rats from the previous study were given *0.50 M NaCl* and water to drink *immediately* after PEG injection. In any case, it is evident that water consumption is not a prerequisite for the development of salt appetite after sc PEG treatment in rats (see also Blackburn et al., 1992).

In explanation, PEG-induced thirst is predominantly driven by baroreceptor-mediated neural signals (Kaufman, 1984; Stricker, 1977), which likely appear before the humoral signals that stimulate PEG-induced salt appetite. The genesis of salt appetite relies largely on the renin-angiotensin-aldosterone system (Fluharty et al., 1983; Stricker, 1983; Stricker et al., 1979). The hormone levels in the blood are not immediately elevated after PEG treatment but increase after several hours and correspond to the appearance of salt appetite (Stricker et al., 1979). Consistent with this idea, PEG-treated rats given pretreatments that increase circulating mineralocorticoids or brain angiotensin II (AngII) show an augmented salt appetite that occurs sooner than it would in PEG-treated rats not given such pretreatments (Stricker, 1983).

Considerable evidence indicates that pituitary OT is secreted in large amounts in response to hypovolemia, and suggests that OT is a potent inhibitory peptide in the central control of salt appetite (Stricker & Verbalis, 1996). For these reasons, it seems paradoxical that the hypovolemic rats consumed 0.30 M NaCl. There are several explanations why elevated pOT

levels might accompany such a large salt appetite. First, whereas pOT is elevated, central secretion of OT (cOT) may not be increased. Of importance is that central, but not peripheral, administration of OT inhibits salt intake (Blackburn et al., 1992; Stricker & Verbalis, 1987). Hypothalamic oxytocinergic magno- and parvocellular neurons have been shown to be co-activated and, for this reason, pOT is often used as a marker for cOT (Stricker & Verbalis, 1996). Second, it is possible that the strength of the excitatory signal associated with the present treatments overrides cOT's inhibitory signal. Blackburn et al. (1992) observed that PEG-treated rats given intraperitoneal injection of hypertonic NaCl solution and central administration of naloxone increased pOT levels from 56.9 pg/ml to 120.4 and 252.0 pg/ml, respectively, and both treatments suppressed salt intake. Therefore when the present PEG-treated rats showed evidence of a salt appetite, the inhibitory signal associated with cOT may be smaller and less potent than the excitatory stimulus.

As mentioned, thirst and salt appetite appear to co-exist in PEG-treated rats during an initial drinking episode. Moreover, because many rats consumed approximately equal volumes of water and salt, the relative magnitudes of thirst and salt appetite appear to alternate while the rats are consuming fluids. That is, consumption of one fluid may inhibit the motivation to continue consuming that fluid and thereby increase the relative motivation to drink the other fluid, ultimately stimulating the animal to switch to that other fluid.

What signals inform the rat when to switch from water to 0.30 M NaCl, and vice versa? Presumably, the motivation to switch fluids is directed by the cumulative magnitudes of excitatory and inhibitory signals of thirst and salt appetite. Several reports have hypothesized that the concentration of fluid emptied from the stomach is detected by nearby osmoreceptors (Choi-Kwon et al., 1990). The apparent anatomical location of these putative visceral receptors

– near the pylorus – allows "sampling" of ingested fluid before it reaches the systemic blood circulation. Such receptors have been implicated in the regulation of water and salt intake, gastric emptying, and VP secretion (Bykowski et al., in press; Smith et al., 2007; Stricker et al., 2007; Manesh et al., 2006; Hoffmann et al., 2006; Stricker & Hoffmann, 2005; Stricker et al., 2002; Tordoff et al., 1987), and they presumably communicate with the brain via vagal and/or splanchnic connections (Carlson et al., 1998; Choi-Kwon et al., 1990; Tordoff et al., 1986). Thus, the concentration of emptied fluid may impact the relative motivations for salt appetite and thirst and determine when the rat will switch. However, in order for visceral osmoreceptors to control which fluid is consumed, there should be temporal juxtaposition between when the rat switches fluid intake and when the fluid emptying from the stomach changes. Yet in these experiments rats sometimes switched several times within one minute even though only subtle changes in concentration of gastric contents occurred (determined by calculation). Thus, it seems unlikely that visceral osmoreceptors are the sole players in sensing and directing when an animal should switch fluids although it remains possible that they do play a role in the process.

An additional possibility is that gustatory receptors mediate the switch in fluid. Ingested fluid is first evaluated when it contacts taste receptors in the mouth. Part of the evaluation consists of assessing whether this fluid is appropriate to the animal's motivation. This assessment provides immediate feedback to the animal and helps the animal decide if it should continue to consume that fluid or not. For example, a water-deprived thirsty animal will drink very little 0.30 M NaCl, whereas the opposite is true in a Na^+-deprived adrenalectomized animal with a large salt appetite. PEG-treated rats evidently enjoy the taste of salty solutions. But what concentration does a rat with a salt appetite prefer? Upon consumption of concentrated saline such as 0.30 M NaCl solution, a salty sensation resides in the mouth presumably for some time

after the rat stops consuming the fluid. Perhaps rats drink water then in order to decrease the intensity of the salty sensation.

Although PEG-treated rats consumed both water and hypertonic saline in an initial drinking episode, they stopped drinking before plasma volume deficits were repaired (Table 1). It has been hypothesized that a pre-systemic inhibitory signal inhibits further fluid consumption (Smith et al., 2007). Thrasher et al. (1981) reported that a pre-systemic signal associated with the act of drinking inhibits thirst and VP secretion in water-deprived dogs before systemic blood was affected. Furthermore, thirst and VP secretion were rapidly inhibited regardless of the concentration of the ingested fluid and whether or not it emptied from the stomach. However, inhibition of thirst by oropharyngeal signals is not present in rats; VP secretion and thirst are not inhibited after ingestion of isotonic saline or when rats drink with an open gastric fistula (Davis et al., 2002; Huang et al., 2000; Waldbillig & Lynch, 1979). Accumulating evidence suggests that pre-systemic inhibitory signals for thirst and salt appetite stem from distension of the stomach and small intestine (Bykowski et al., in press; Hoffmann et al., 2006; Smith et al., 2007; Stricker et al., 2007).

4.2 GASTRIC EMPTYING

Multiple factors appeared to influence the rate of gastric emptying in the present experiments, some directly and some indirectly. One factor is the drinking rate (Fig. 4). We know from numerous previous observations in this laboratory that rats empty ingested fluid much more slowly while not drinking than while drinking. In the present experiment, drinking rates varied greatly (0.4 – 1.5 ml/min) and were inversely proportional to hypovolemia (not

shown). Rats paused more frequently and for longer durations the more hypovolemic they were, probably due to hypovolemia-induced lethargy. The faster the fluid was consumed, the faster it emptied, though there were differences between subgroups based on the concentration of the fluid consumed. It seems that inhibition of gastric emptying associated with hypertonicity of the ingested fluid – as detected by gustatory and visceral osmoreceptors – blunts the effects of fast drinking rate.

When rats consumed water and 0.30 M NaCl in the same drinking episode, emptying was often faster than emptying of 0.30 M NaCl alone. However, the four rats that consumed little water in a two-bottle test emptied much less than the other rats that drank the two fluids, and emptied similarly to rats that consumed 0.30 M NaCl alone (Fig. 4). Hence, simply alternating between fluids does not ensure increased gastric emptying; emptying is related to the concentration that contacts gustatory and visceral osmoreceptors, and is facilitated when the two fluids are consumed in approximately equal proportions.

Many reflexes exist that are involved in the ingestive and digestive processes of foods and fluids – originating from the entire length of the alimentary canal (i.e., from the mouth to the anus) (Ferreira et al., 2005; Goyal et al., 2001; Kobashi et al., 2000). Pyloric sphincter tone plays a crucial role in the control of gastric emptying. It is conceivable that pyloric sphincter tone is influenced by an oropharyngeal signal associated with taste or swallowing. In particular, the correlation between drinking and emptying rate may be mediated by an effect of swallowing rate on pyloric tone. Pharyngeal sensory afferents are well-characterized in the inhibition of drinking and VP secretion in dogs, sheep, and humans (Figaro & Mack, 1997; Geelen et al., 1984; Thrasher et al., 1981), though not in rats (Huang et al., 2001). Nonetheless, it is plausible that a reflex relationship exists between the pharynx and/or esophagus and pyloric sphincter.

Specifically, pharyngeal or esophageal stimulation may affect the frequency, length of time, and/or diameter at which the pylorus opens.

It is possible that emptying rate determines drinking rate, rather than the opposite being true. Substantial evidence suggests that gastric distension is an inhibitory signal for food and fluid intake in rats (Curtis & Stricker, 1997; Davis & Campbell, 1973). Thus, the faster fluid is emptied from the stomach, the less gastric distension present, the less inhibition of thirst and salt appetite, and the more fluid is consumed.

A high drinking rate can be advantageous to a rat with body fluid deficits. In order to quickly quench an animal's thirst and quell an animal's physiological deficits, ingested fluid must be consumed quickly and be delivered into the systemic circulation quickly. Gastric emptying and drinking rates are related to each other, but both are also inversely related to the degree of hypovolemia. In addition to the possibility that drinking rate directly influences gastric emptying rate, it is possible that the relationship exists between these two variables because each is influenced by a third variable. Hypovolemia is a potent stimulus of the sympathoadrenal system, activation of which is associated with decreases in gastric emptying, GI motility, and blood flow to the GI tract (Stebbing et al., 2001; Spencer et al., 1999). Thus, the more hypovolemic a rat is, the less able they should be to empty ingested fluid. In fact, PEG-treated rats appear to empty ingested fluid maximally until they become very hypovolemic (i.e., there is increasing conflict between tendency of ingested fluids to empty quickly because of fast drinking rate and to empty slowly because of sympathetic inhibition of gastric emptying).

As stated, gastric emptying decreased in proportion to plasma protein concentration, confirming the observations of Smith et al. (2007). Because drinking rate and emptying rate are related, hypovolemia might not be causally related to emptying rate, but may be secondary to the

relationship between drinking rate and hypovolemia. Thus, it is possible that the negative correlation between emptying rate and plasma protein concentration is a result of the negative correlation between drinking rate and plasma protein, and that gastric emptying is not affected by drinking rate or taste alone but by a combination of variables. The fact that many factors contribute to the control of gastric emptying is likely responsible for the variability in the data presented here.

Like thirst and salt appetite, gastric emptying of water and NaCl solutions is influenced by pre-systemic factors. For example, emptying is slowed when hypertonic NaCl solutions are emptied even before pNa is affected. Furthermore, hypertonic NaCl solutions empty at a rate that is inversely proportional to its hypertonicity and, consequently, the rate at which meq of Na^+ are emptied is constant (Bykowski et al., in press; Stricker et al., 2007). The pre-systemic concentration-dependent control of gastric emptying is likely mediated through visceral osmoreceptors. As hypertonic saline empties from the stomach and contacts visceral osmoreceptors, gastric emptying is inhibited and thereby decreases further emptying of that hypertonic solution. Visceral osmoreceptors can also excite or inhibit thirst and salt appetite. Thus, this feed-forward mechanism can elicit appropriate physiological and behavioral responses to repair or prevent perturbations (or further aggravation) of pOsm.

PEG-treated rats empty 0.30 M NaCl much more slowly than 0.15 M NaCl (Smith et al., 2007). In Fig. 5, the regression line for rats that drank 0.15 M NaCl is essentially parallel to the "empty everything" line. Rats empty little fluid at the beginning of a drinking episode. During this time ~2 ml of ingested fluid accumulates in the stomach and a small amount is emptied into the small intestine to allow "sampling" by visceral osmoreceptors. If the fluid is hypertonic, rats

continue emptying at a very slow rate, whereas rats empty essentially all of the fluid (as indicated by the line parallel with the "empty everything" line) when the fluid is 0.15 M NaCl.

In the present two-bottle tests, however, ingestion of water greatly facilitated gastric emptying of 0.30 M NaCl, regardless of which fluid was consumed first (Fig. 5). Thus, the fluid that initially emptied into the small intestine did not set the emptying rate for very long, but rather gastric emptying was dynamically regulated. The ingestion of water subsequent to 0.30 M NaCl dilutes the fluid that suffuses visceral osmoreceptors and thereby decreases the hypertonicity-mediated inhibition of gastric emptying. Ingestion of water did not facilitate emptying when it was consumed in amounts much smaller than the volume of 0.30 M NaCl ingested (Fig. 5, four encircled points). The concomitant thirst and salt appetite are useful in that they potentiate delivery of the ingested fluid through the GI tract and, ultimately, into the general blood circulation. Maintaining rats on a NaD diet, itself, did not seem to affect gastric emptying (Fig. 5).

Because these receptors have been implicated to be near the pylorus, we assumed the concentration of gastric contents was similar to the concentration being emptied from the stomach and contacting visceral osmoreceptors. As Fig. 6 shows, gastric emptying rate decreased in proportion to the concentration of fluid emptied from the stomach. A linear regression line was not included in Fig. 6 because without the five points to the right of 154 mM the data are not statistically significant. The variability to the left of 154 mM does not support a linear regression line in which hypotonic fluid empties faster than isotonic which empties faster than hypertonic solution. In fact, other studies suggest that isotonic NaCl solution empties faster than water when ingested by rats (Smith et al., 2007; Stricker et al., 2007).

Gastric emptying of hypertonic saline slowly delivers hypertonic fluid into the small intestine. In this way, sudden elevations of pOsm are prevented. Additionally, hyperosmolality-induced inhibition of gastric emptying serves as a protective mechanism against osmotic diarrhea. Osmotic diarrhea occurs in response to a large osmotic load within the intestinal lumen – as would occur with rapid gastric emptying of hypertonic fluid – pulling water into the intestinal lumen, resulting in excessive loss of water and electrolytes in the stool. It is important that detection of the hypertonic fluid occurs before substantial amounts are emptied into the intestinal lumen. Cerebral osmoreceptors are not anatomically situated to detect elevations of small intestinal lumen osmolality, and thus they are likely not responsible for mediating the decreased gastric emptying. More likely, a local, pre-systemic signal exists to prevent osmotic diarrhea – presumably mediated through visceral osmoreceptors.

Taste has been implicated to affect gastric motility in rats. In particular, Kobashi et al. (2000) showed that gastric motility was highest when 0.15 M NaCl was applied to the larynx and epiglottis of anesthetized rats and it decreased in proportion to the hypo- or hypertonicity of the administered fluid. Gastric motility plotted as a function of the concentration administered resulted in an inverted "V-shaped" curve, in which 0.15 M NaCl was associated with the peak of the curve. It seems likely that taste and laryngeal signals provide another early influence on gastric emptying when rats consume NaCl solutions.

To summarize, it is clear that the control of gastric emptying of water and NaCl solutions in PEG-treated rats is complex and influenced by multiple factors, namely: drinking rate, taste, degree of hypovolemia, and visceral osmo-detection.

4.3 ABSORPTION FROM THE SMALL INTESTINE

Ingested fluid does not immediately restore plasma volume deficits of PEG-treated rats. Restoration of plasma volume deficits is contingent on fluid movement through the GI tract and into the systemic circulation. Such movement of ingested fluid is controlled by gastric emptying and by small intestinal absorption. PEG-treated rats readily absorb water and 0.15 M NaCl from the small intestine but they absorb 0.30 M NaCl very slowly (Smith et al., 2007). In fact, in that recent study three rats that were killed 15 – 30 min after they started consuming 0.30 M NaCl showed essentially no net absorption of ingested fluid from the small intestine. These rats absorbed little in part because they emptied little fluid from their stomachs, but primarily because hypertonic saline solution was present within the small intestinal lumen. Hypertonic saline generates an osmotic gradient that pulls interstitial water into the lumen (Follansbee, 1945), resulting in little net absorption. In contrast, PEG-treated rats absorb ~4.6 ml of mixed fluid by 15 min after ingestion (Fig. 7).

Based on the equation, [(plasma protein$_2$ – plasma protein$_1$) x 100/ plasma protein$_2$], the intravascular fluid volume deficit can be calculated after sc PEG treatment (Stricker, 1968). Animals injected sc with 30% PEG and killed 16 h later without having had access to drinking fluids had a plasma protein concentration of 9.0 g/dl and an estimated 32% decrease in plasma volume, which is ~4.6 ml in a 375 g rat. On average, rats that drank water and 0.30 M NaCl had a net absorption of ~3.1 ml in 12 min (Fig. 7), which – if all of it remained in the circulation – would repair 67% of the blood volume deficit. However, pProt values of PEG-treated rats drinking both fluids were ~8.3 g/dl, which suggests that they had a 26.5% plasma volume deficit and therefore experienced only ~17% restoration of the deficit. Thus, most of the ingested fluid probably went into the sc edema. In an earlier study of PEG-treated rats given access to drinking

fluids immediately after injection, much of the ingested fluid also was found to be sequestered in the edema site rather than staying in the intravascular space (Stricker & Jalowiec, 1970).

To maintain body fluid homeostasis, a rat with plasma volume deficits needs to deliver ingested fluid into the systemic circulation. Absorption of 0.30 M NaCl occurs very slowly. On the other hand when 0.30 M NaCl is ingested with water, emptying and absorption are greatly potentiated (Fig. 7). For these reasons, it is clearly beneficial to the PEG-treated rats that they consumed the two fluids in proportions that often approximated the concentration of body fluids, a concentration that moves most quickly through the GI tract and is optimal for repairing body fluid volume deficits without disturbing pOsm.

Another benefit of concurrent thirst and salt appetite is the effect of rapid gastric emptying and intestinal absorption of ingested water and 0.30 M NaCl on further fluid intake. Because distension of the stomach and small intestine inhibits consumption of both water and saline (Bykowski et al., in press; Smith et al., 2007; Stricker et al., 2007; Hoffmann et al., 2006), the faster the fluid is transported out of the GI tract, the less distension occurs, the less inhibition of thirst and salt appetite there is, and the more of each fluid the rats consume. Accordingly, the accelerated movement of ingested fluid through the GI tract may be, in part, the reason why rats drink more when they drink both fluids than when they drink 0.30 M NaCl alone.

PEG-induced hypovolemia is a substantial stimulus for renin secretion (Stricker et al., 1979). Renin is converted to the peptide AngII through a short enzymatic cascade. AngII is crucial for multiple aspects of cardiovascular maintenance including hormonal actions to stimulate thirst and salt appetite, renal reabsorption of Na^+ (and water), and vasoconstriction. Additionally, it has been proposed to increase small intestinal absorption – a process that complements AngII's other actions to increase and maintain blood volume and pressure.

Endogenous AngII has been shown to increase water and Na^+ absorption from the small intestine after hemorrhage, an effect that is abolished by bilateral nephrectomy or peripheral injection of the ACE inhibitor, captopril (Levens, 1984; Levens et al., 1984). Moreover, AngII binding sites and specific AngII receptor subtypes have been identified in the GI tract (Duggan, 1989; Jin 1998). Alternatively (or additionally), AngII may increase absorption indirectly via its effect to stimulate secretion of VP or aldosterone. Thus, increased fluid absorption from the small intestine may be an adaptive regulatory response to hypovolemia. Furthermore, the more hypovolemic a rat is, the more efficient absorption from the small intestine might be.

4.4 SUMMARY

Detection of blood volume deficits alters a rat's motivational state by stimulating thirst and salt appetite. In consequence, PEG-treated rats with established hypovolemia drink both water and hypertonic NaCl solution; indeed, they quickly alternate between drinking both fluids and concoct a mixture isotonic to body fluids – a concentration that is most effective in repairing plasma volume deficits without perturbing pOsm. However, their plasma volume deficits cannot be restored until ingested fluid is absorbed from the GI tract. In fact, ingestion of both water and 0.30 M NaCl accelerates fluid delivery into the systemic circulation. In particular, concurrent consumption of water and 0.30 M NaCl augments the rate of gastric emptying and small intestinal absorption. Moreover, as a consequence of fluid leaving the GI tract more quickly, GI distension signals associated with inhibition of fluid intake are quickly removed, leading to larger fluid intakes. The unique behavior of PEG-treated rats allows restoration of their body fluid deficits in association with complementary physiological and hormonal contributions to

body fluid homeostasis. Clearly, co-existence of thirst and salt appetite is an adaptive behavioral response to hypovolemia.

APPENDIX A

Table 1: ***Uninterrupted PEG-treated rats drinking water and 0.30 M NaCl in a two-bottle drinking test***

	n	# of bouts	avg. bout size (min)	time kill (min)	total intake (ml)
saline first	11	5.3 ± 1.0[a]	2.2 ± 0.3	10.4 ± 1.4	9.1 ± 0.9
water first	4	2.5 ± 0.3	3.9 ± 0.7	9.9 ± 1.5	8.0 ± 1.2
NaD diet	10	4.0 ± 0.5[a]	3.4 ± 0.7	10.7 ± 1.2	11.2 ± 1.3

Mean ± SE values are shown. All PEG-treated rats were given a two-bottle test with water and 0.30 M NaCl, and they drank without interruption until they stopped. Some rats were maintained on sodium-deficient (NaD) diet for 4 days before testing, all others were maintained on standard chow.

[a] $P < 0.05$ in comparison to "water first"

FIGURE LEGENDS

Fig. 1. Drinking pattern of an individual rat 16 h after sc PEG treatment. This animal had 9 bouts in an initial drinking episode. The rapid alternation between water and 0.30 M NaCl consumption suggests concurrent thirst and salt appetite.

Fig. 2. Cumulative 0.30 M NaCl intake plotted as a function of cumulative water intake during the initial drinking episode. Shown are intakes by rats that consumed (A) saline first, (B) water first, or (C) rats that had been maintained on NaD diet for 4 days prior to the experiment. Symbols represent data collected from individual animals. The solid, diagonal black line in each figure shows intakes of water and 0.30 M NaCl in equal amounts, resulting in a 0.15 M NaCl mixture. The solid gray line in Fig. 2A shows the rat from Fig. 1. Below and to the left of each symbol is a dashed line that shows the individual bouts of water and 0.30 M NaCl. The encircled symbols in Figs. 2A (3 symbols) and 2C (1 symbol) represent rats that consumed mostly 0.30 M NaCl; data from those four animals are again encircled in subsequent figures. Note that the same axes were used in each figure. Not shown are the data from the only two rats whose intakes were too large to be contained within the axes. Both rats had been maintained on NaD diet before PEG treatment (Fig. 2C); one animal drank 9.8 ml saline and 5.5 ml of water while the other drank 6.3 ml saline and 14.5 ml of water.

Fig. 3. Cumulative intakes of water and NaCl solution by PEG-treated rats during the initial drinking episode, plotted as a function of time when the rats were killed. Each symbol represents data collected from an individual animal. Data are from the same animals as in Figs. 2A-2C. The dashed line represents the regression line for rats that drank only water, 0.15 M NaCl, or 0.30 M NaCl in one-bottle tests; data were re-analyzed from C.A Smith's master's thesis and shown for purposes of comparison (individual data points not shown). The equation of the regression line is $y = 0.766x + 2.128$. Data from the present two-bottle drinking tests generally correspond to that line, although data from three of the four rats that drank mostly

saline (encircled symbols) were among the exceptions. The high correlation between fluid intake and drinking time ($r = 0.75$; $P < 0.01$) suggests a steady rate of intake by these animals.

Fig. 4. Gastric emptying rate of fluid ingested during the initial drinking episode, plotted as a function of drinking rate. Each symbol represents data collected from an individual animal. Data are from the same animals as in the previous figures. The solid line indicates identical rates of gastric emptying and drinking ($y = x$) and is displayed for purposes of comparison. The dashed line represents the regression line for saline-first and water-first rats, as well as for NaD diet rats ($y = 0.4933x + 0.0703$). In general, drinking rate was slower than but linearly related to gastric emptying rate ($r = 0.75$, $P < 0.01$). The encircled symbols indicate rats that consumed very little water (see Figs. 2A and 2C) and emptied at a slow rate resembling that of PEG-treated rats drinking 0.30 M NaCl only (Smith et al., 2007).

Fig. 5. Volume of ingested fluid that emptied from the stomach during the initial drinking episode, plotted as a function of cumulative intake of water and 0.30 M NaCl. Each symbol represents data collected from an individual animal. Data are from the same animals as in the previous figures. The solid line represents the situation if all of the ingested fluid was emptied from the stomach ($y = x$). Data from Smith et al. (2007) were used to calculate the regression lines (data not shown): for 0.15 M NaCl (*upper line*), $y = 0.9191x - 1.5378$; for 0.30 M NaCl (*lower line*), $y = 0.3262x + 0.328$. These lines are shown for purposes of comparison. Rats that drank both water and 0.30 M NaCl usually emptied more of the ingested fluid than rats that drank 0.30 M NaCl alone; in fact, they often emptied amounts similar to those of rats that

drank 0.15 M NaCl alone. Notable exceptions are the four rats that drank the most concentrated fluid mixtures (encircled symbols, as in previous figures).

Fig. 6. Rate at which ingested fluid emptied from the stomach during the initial drinking episode, plotted as a function of the concentration of fluid in the stomach. Each symbol represents data collected from an individual animal. Data are from the same animals as in the previous figures. Gastric emptying rates were fastest when the stomach concentration was 154 mM NaCl and below, whereas the slowest rates were associated with the highest NaCl concentrations in the stomach.

Fig. 7. Net volume of ingested fluid absorbed from the small intestine during the initial drinking episode, plotted as a function of the time that rats were killed after drinking began. Each symbol represents data collected from an individual animal. Data are from the same animals as in the previous figures. Regression equation for saline-first, water-first, and NaD diet rats: $y = 0.3066x - 0.6249$. Net volume of fluid absorbed from the small intestine increased with time except when rats drank mostly 0.30 M NaCl. The correlation coefficient ($r = 0.82$, $P < 0.001$) does not include the four animals (encircled symbols, see Figs. 2A and 2C) that drank the most concentrated fluid mixtures; those rats had very little net absorption, like PEG-treated rats drinking 0.30 M NaCl only (Smith et al., 2007).

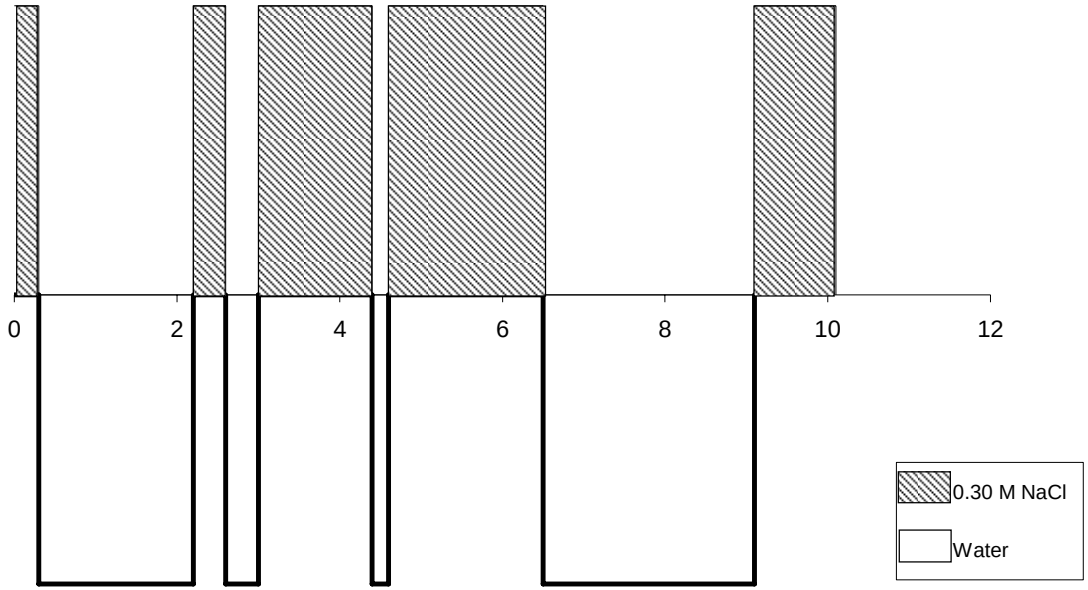

Figure 1

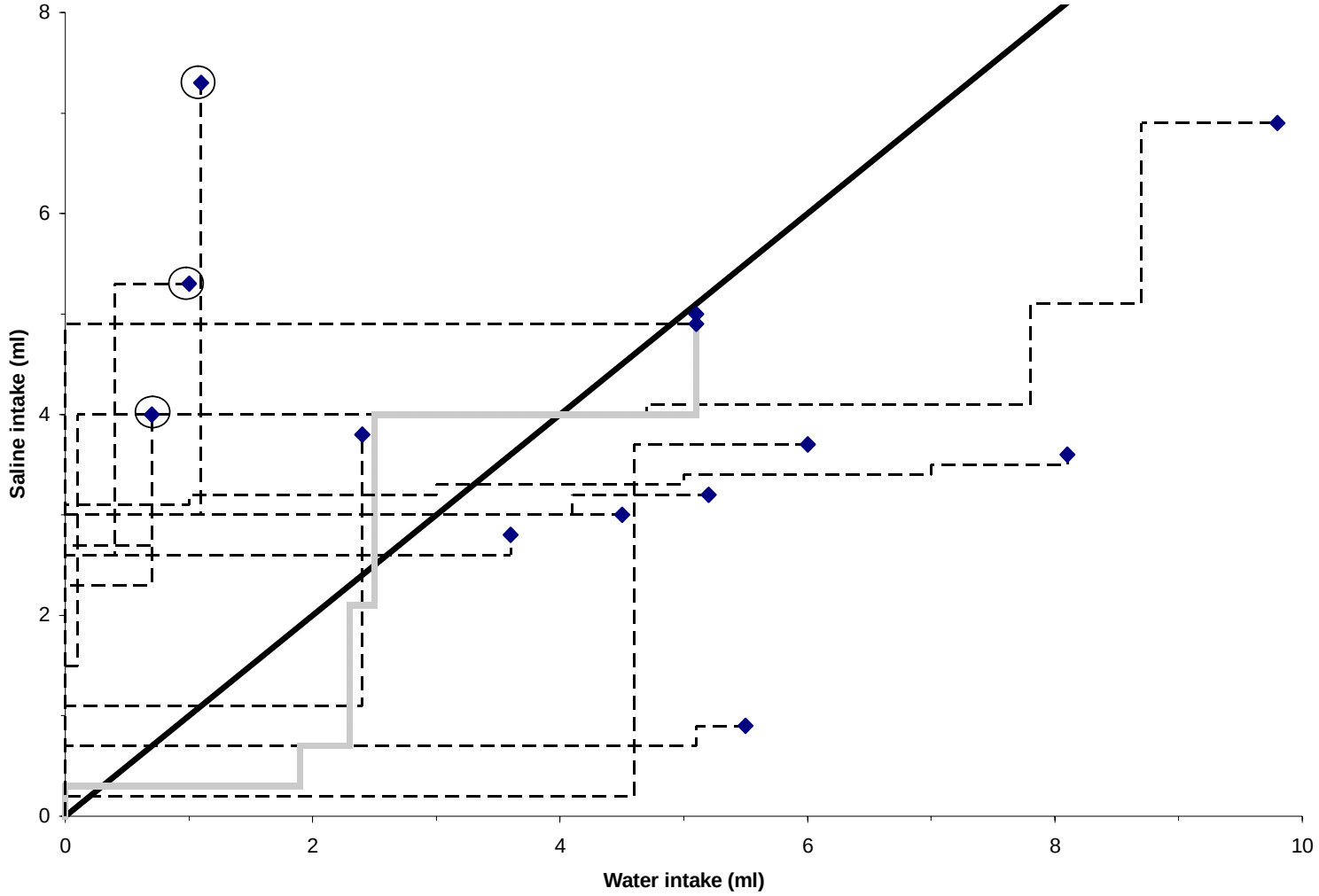

Figure 2A

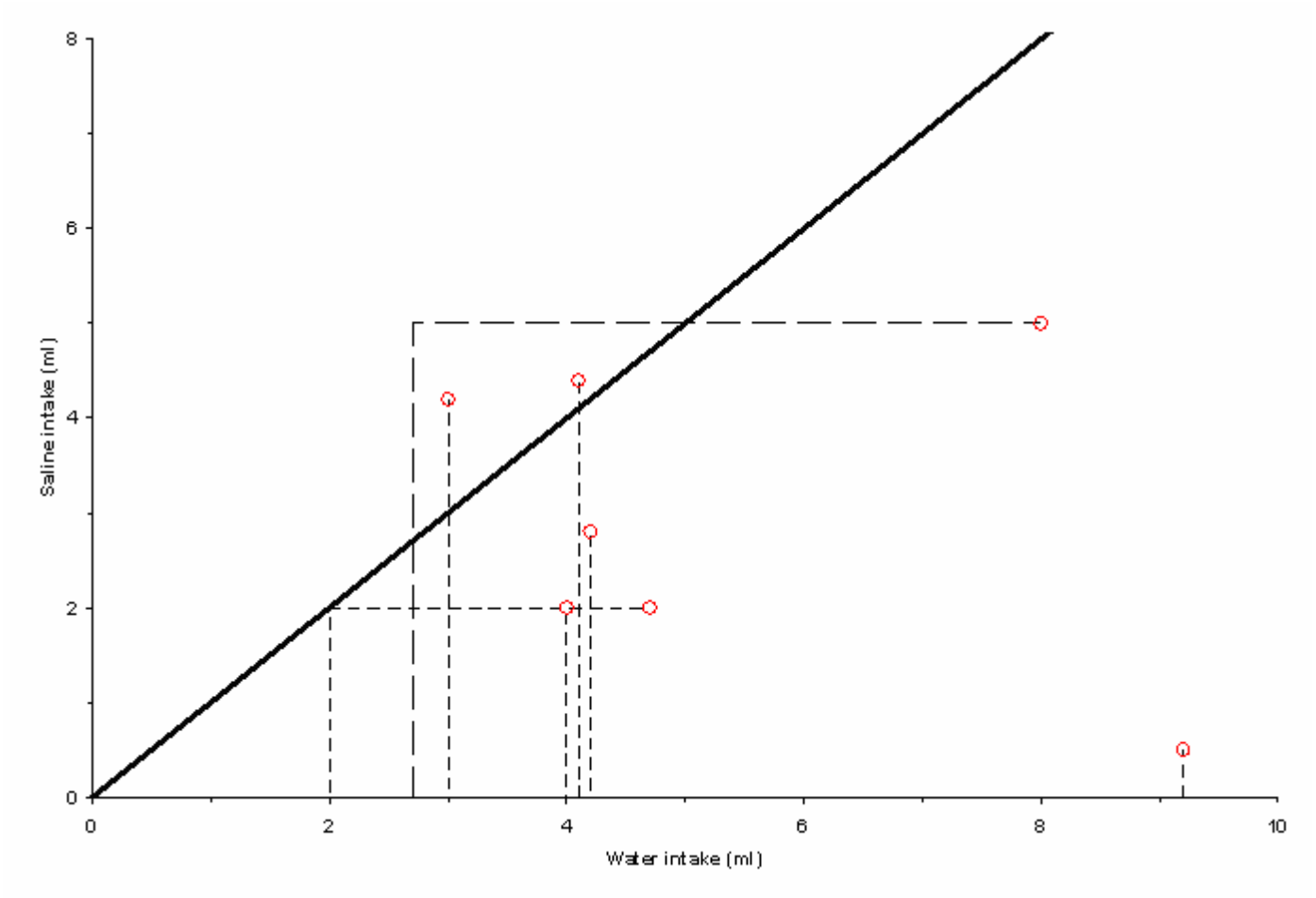

Figure 2B

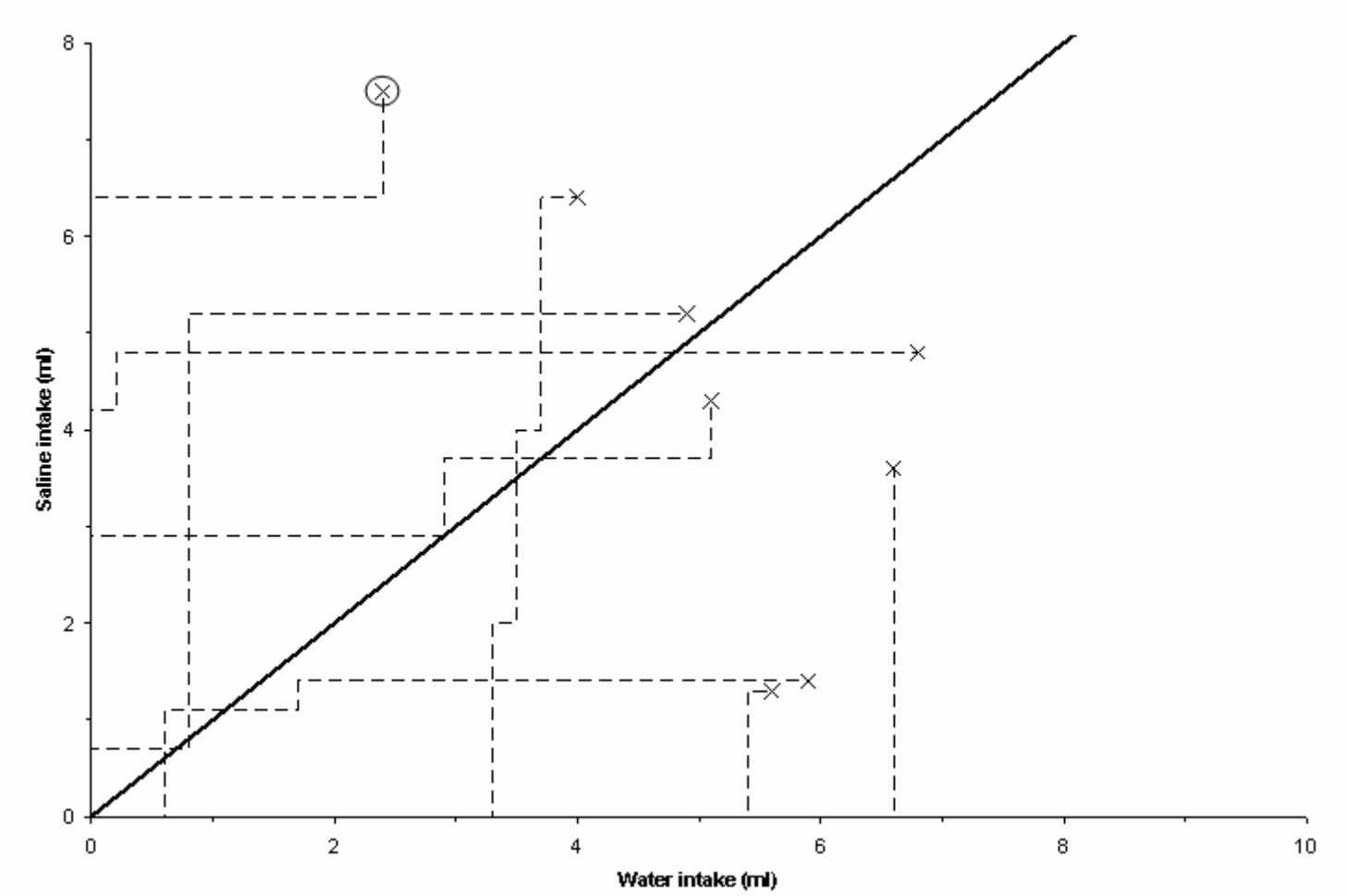

Figure 2C

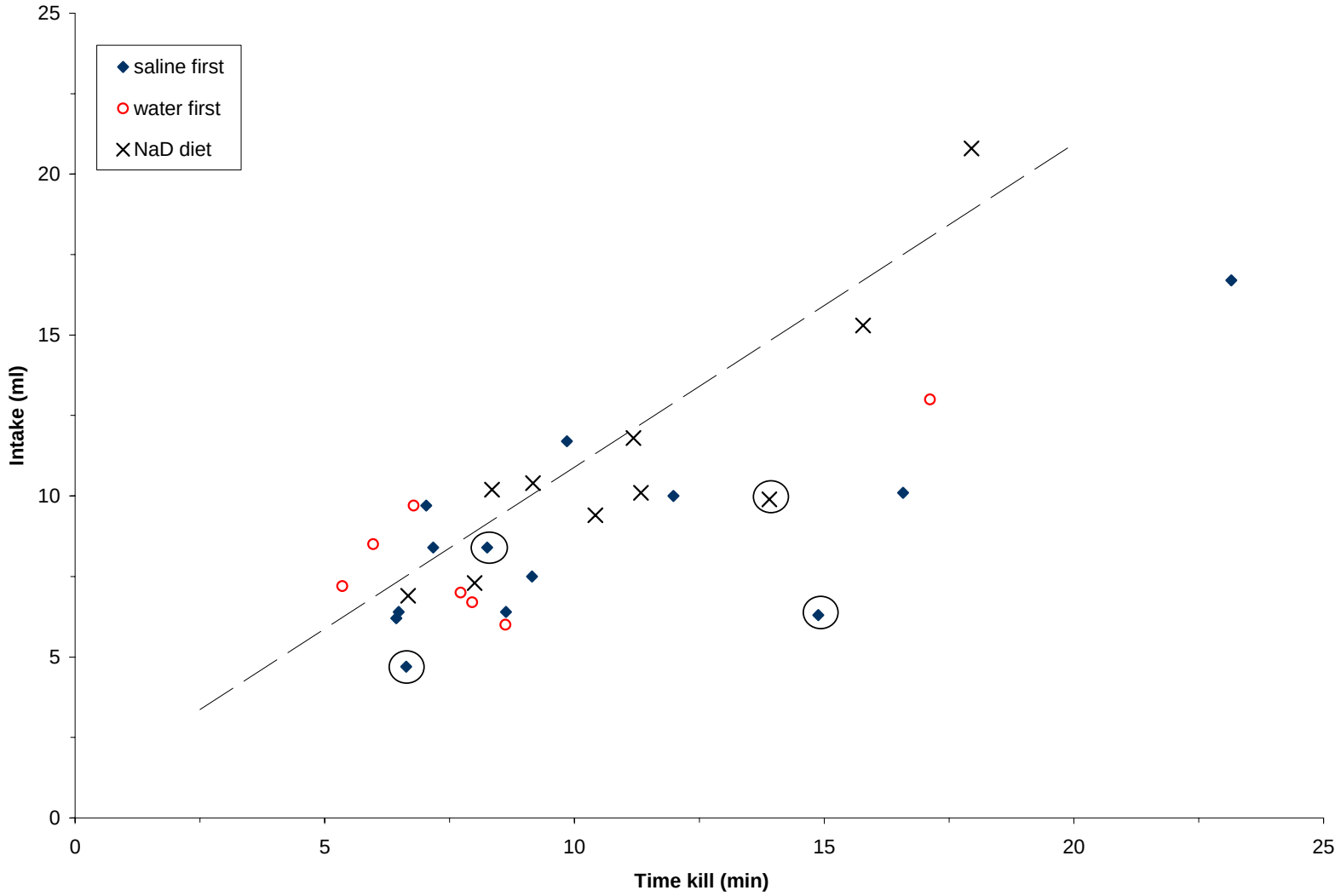
saline first
water first
NaD diet
Intake (ml)
Time kill (min)
0
5
10
15
20
25

Figure 3

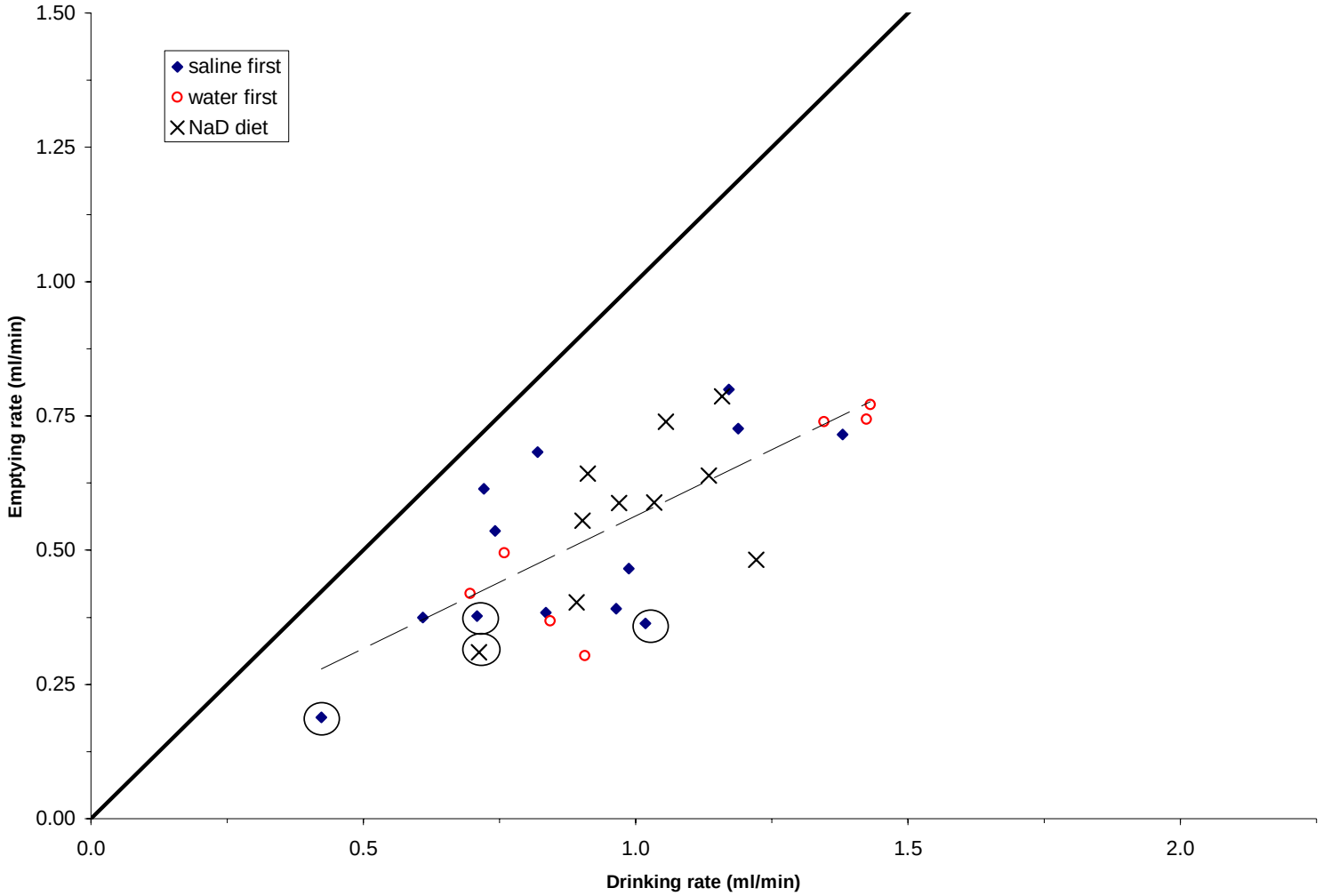
saline first
water first
NaD diet
Emptying rate (ml/min)
Drinking rate (ml/min)
0.00
0.25
0.50
0.75
1.00
1.25
1.50
0.0
0.5
1.0
1.5
2.0

Figure 4

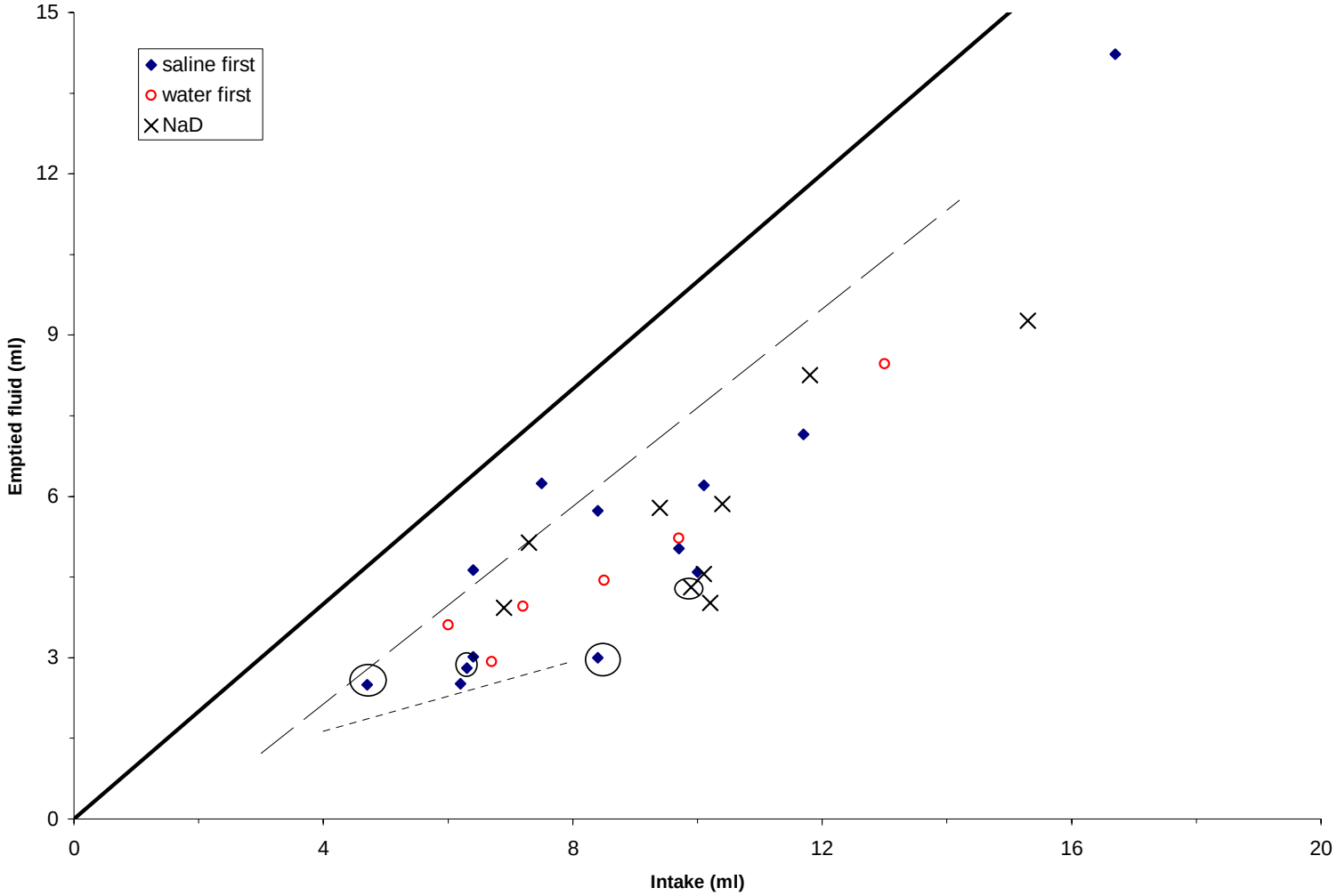
saline first
water first
NaD
Emptied fluid (ml)
Intake (ml)
0
3
6
9
12
15
0
4
8
12
16
20

Figure 5

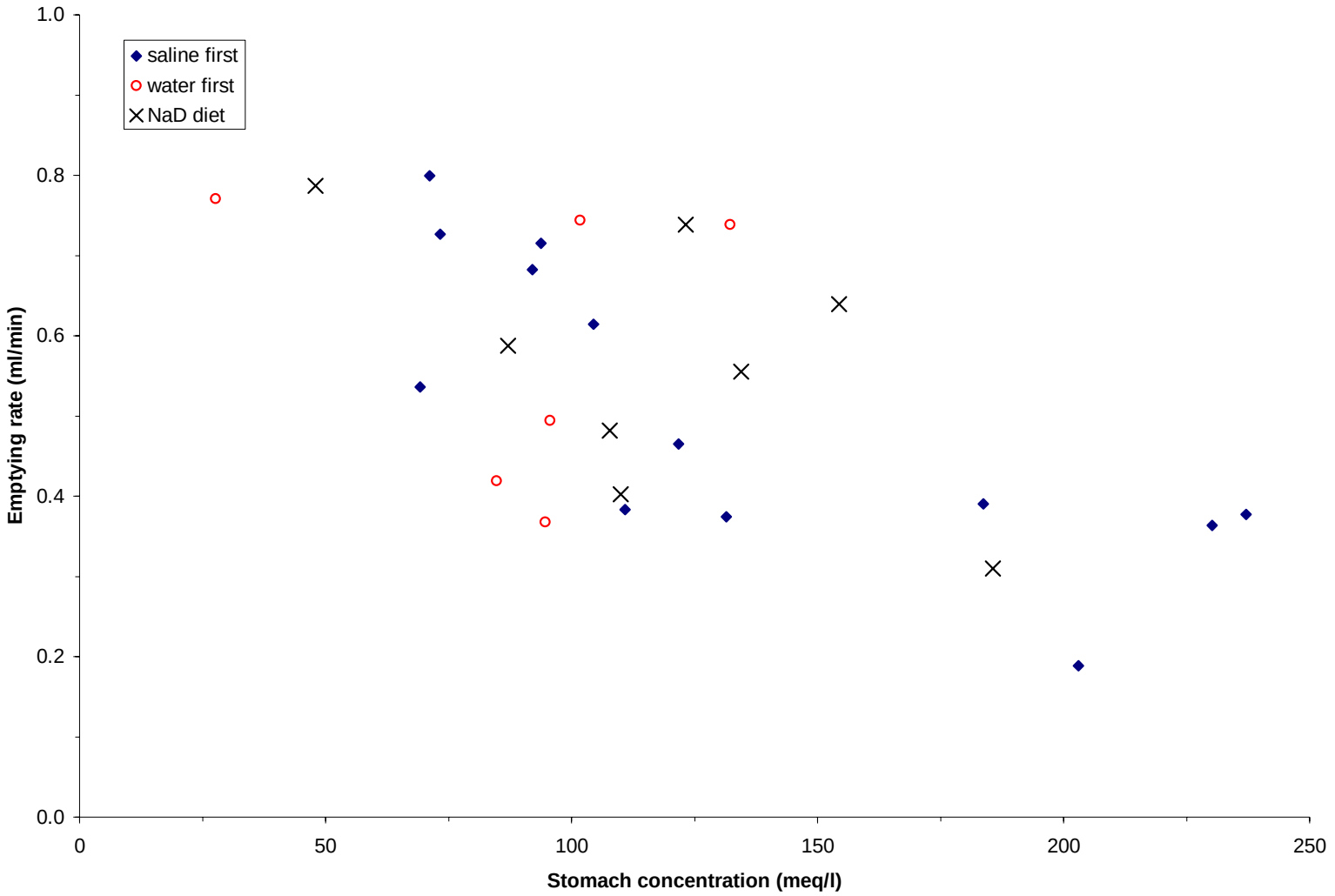
saline first
water first
NaD diet
Emptying rate (ml/min)
1.0
0.8
0.6
0.4
0.2
0.0
0
50
100
150
200
250
Stomach concentration (meq/l)

Figure 6

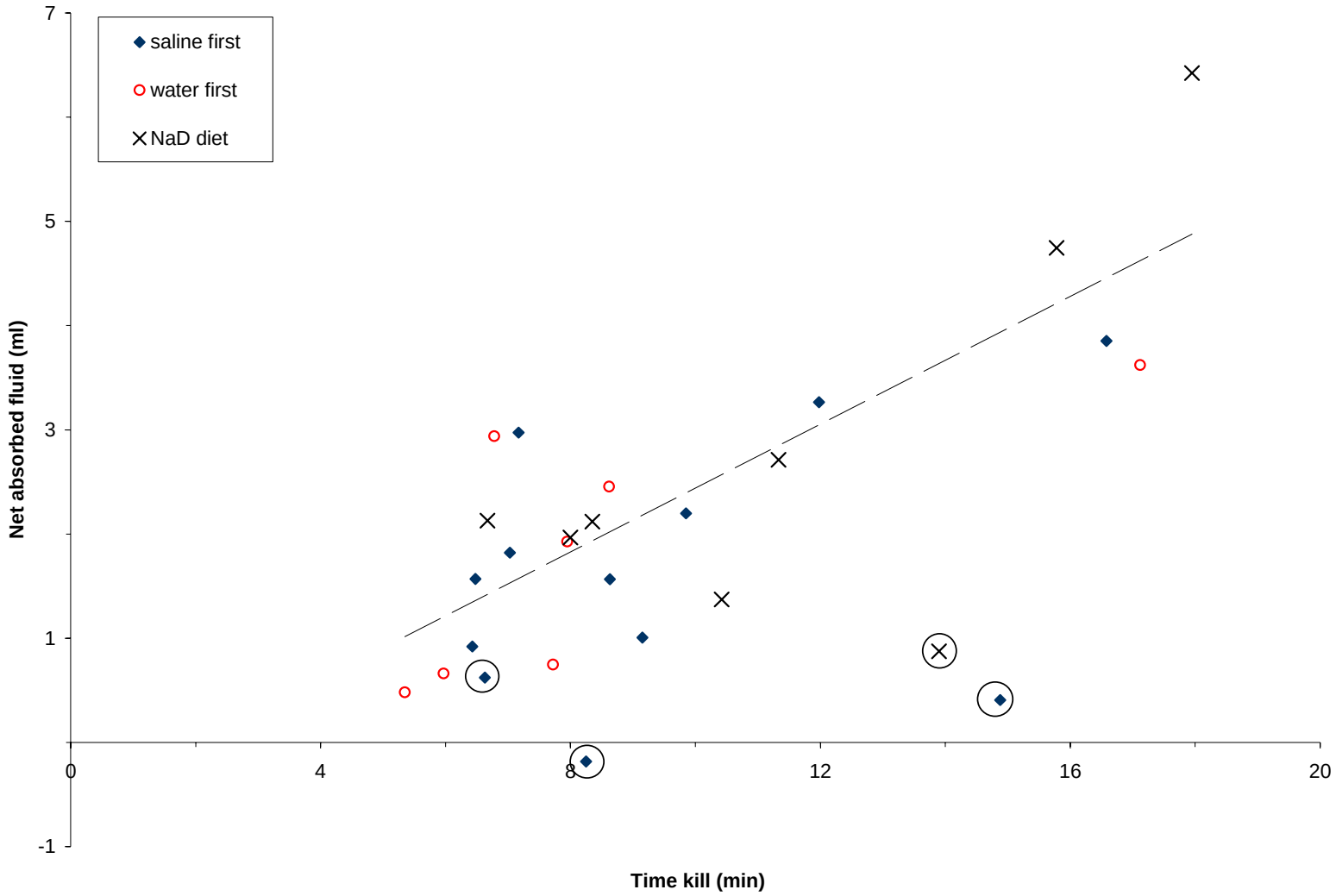
saline first
water first
NaD diet
Net absorbed fluid (ml)
Time kill (min)
7
5
3
1
-1
0
4
8
12
16
20

Figure 7

REFERENCES

Blackburn RE, Stricker EM, and Verbalis JG. Central oxytocin mediates inhibition of sodium appetite by naloxone in hypovolemic rats. *Neuroendocrinology* 56: 255-263, 1992.

Bykowski MR, Smith JC, and Stricker EM. Regulation of NaCl solution intake and gastric emptying in adrenalectomized rats. *Physiol Behav*, in press, 2007.

Carlson SH and Osborn JW. Splanchnic and vagal denervation attenuate central Fos but not AVP responses to intragastric salt in rats. *Am J Physiol* 274: R1243-R1252, 1998.

Choi-Kwon S, McCarty R, and Baertschi AJ. Splanchnic control of vasopressin secretion in conscious rats. *Am J Physiol Endocr Metab* 259: E19-E26, 1990.

Curtis KS and Stricker EM. Enhanced fluid intake by rats after capsaicin treatment. *Am J Physiol Regulatory Integrative Comp Physiol* 272: R704-R709, 1997.

Curtis KS, Verbalis JG, and Stricker EM. Area postrema lesions in rats appear to disrupt rapid feedback inhibition of fluid intake. *Brain Res* 726: 31-38, 1996.

Davis JD and Campbell CS. Peripheral control of meal size in the rat: Effect of sham feeding on meal size and drinking rate. *J Comp Physiol Psychol* 83: 379-387, 1973.

Davis JD, Smith GP, and McCann DP. The control of water and sodium chloride intake by postingestional and orosensory stimulation in water-deprived rats. *Physiol Behav* 75: 7–14, 2002.

Duggan KA, Mendelsohn FA, and Levens NR. Angiotensin receptors and angiotensin I-converting enzyme in rat intestine. *Am J Physiol* 257: G504-G510, 1989.

Ferreira M Jr, Sahibzada N, Shi M, Niedringhaus M, Wester MR, Jones AR, Verbalis JG, and Gillis RA. Hindbrain chemical mediators of reflex-induced inhibition of gastric tone produced by esophageal distension and intravenous nicotine. *Am J Physiol Regul Integr Comp Physiol* 289: R1482-R1495, 2005.

Figaro MK and Mack GW. Regulation of fluid intake in dehydrated humans: role of oropharyngeal stimulation *Am J Physiol Regulatory Integrative Comp Physiol* 272: R1740-R1746, 1997.

Fluharty SJ and Epstein AN. Sodium appetite elicited by intracerebroventricular infusion of angiotensin II in the rat: II. Synergistic interaction with systemic mineralocorticoids. *Behav Neurosci* 97: 746-758, 1983.

Follansbee R. The osmotic activity of gastrointestinal fluids after water ingestion in the rat. *Am J Physiol* 144: 355–362, 1945.

Geelen G, Keil LC, Kravik SE, Wade CE, Thrasher TN, Barnes PR, Pyka G, Nesvig C, and Greenleaf JE. Inhibition of plasma vasopressin after drinking in dehydrated humans. *Am J Physiol Regulatory Integrative Comp Physiol* 247: R968-R971, 1984.

Geerling JC, Engeland WC, Kawata M, and Loewy AD. Aldosterone target neurons in the nucleus tractus solitarius drive sodium appetite. *J Neurosci* 26: 411-417, 2006.

Geerling JC and Loewy AD. Aldosterone-sensitive NTS neurons are inhibited by saline ingestion during chronic mineralocorticoid treatment. *Brain Res* 1115: 54-64, 2006.

Geerling JC and Loewy AD. Sodium depletion activates the aldosterone-sensitive neurons in the NTS independently of thirst. *Am J Physiol Regul Integr Comp Physiol* 292: R1338-R1348, 2007.

Goyal RK, Padmanabhan R, and Sang Q. Neural circuits in swallowing and abdominal vagal afferent-mediated lower esophageal sphincter relaxation. *Am J Med* 111: 95S–105S, 2001.

Hoffmann ML, DenBleyker M, Smith JC, and Stricker EM. Inhibition of thirst when dehydrated rats drink water or saline. *Am J Physiol Regulatory Integrative Comp Physiol* 290: R1199-R1207, 2006.

Huang W, Sved AF, and Stricker EM. Water ingestion provides an early signal inhibiting osmotically stimulated vasopressin secretion in rats. *Am J Physiol Regulatory Integrative Comp Physiol* 279: R756-R760, 2000.

Jin XH, Wang ZQ, Siragy HM, Guerrant RL, and Carey RM. Regulation of jejunal sodium and water absorption by angiotensin subtype receptors. *Am J Physiol Regulatory Integrative Comp Physiol* 275: R515-523, 1998.

Kaufman S. Role of right atrial receptors in the control of drinking in the rat. *J Physiol* 349:389-396, 1984.

Kobashi M, Mizutani M, and Matsuo R. Water stimulation of the posterior oral cavity induces inhibition of gastric motility. *Am J Physiol Regulatory Integrative Comp Physiol* 279: R778-R785, 2000.

Levens NR. Modulation of jejunal ion and water absorption by endogenous angiotensin after hemorrhage. *Am J Physiol* 246: G634-G643, 1984.

Levens NR, Marriscotti SP, Peach MJ, Munday KA, and Carey RM. Angiotensin II mediates increased small intestinal fluid absorption with extracellular volume depletion in the rat. *Endocrinology* 114: 1692-1701, 1984.

Manesh R, Hoffmann ML, and Stricker EM. Water ingestion by rats fed a high-salt diet may be mediated, in part, by visceral osmoreceptors. *Am J Physiol Regulatory Integrative Comp Physiol* 290: R1742-R1749, 2006.

Sawchenko PE and Swanson LW. Immunohistochemical identification of neurons in the paraventricular nucleus of the hypothalamus that project to the medulla or to the spinal cord in the rat. *J Comp Neurol* 205: 260-272, 1982.

Smith CA, Curtis KS, Smith JC, and Stricker EM. Presystemic influences on thirst, salt appetite, and vasopressin secretion in the hypovolemic rat. *Am J Physiol Regul Integr Comp Physiol* 292: R2089-R2099, 2007.

Spencer N, McCarron SL, and Smith TK. Sympathetic inhibition of ascending and descending interneurons during the peristaltic reflex in the isolated guinea-pig distal colon. *J. Physiol. (Lond.)* 519: 539–550, 1999.

Stebbing MJ, Johnson PJ, Vremec MA, and Bornstein JC. Role of α_2-adrenoceptors in the sympathetic inhibition of motility reflexes of guinea-pig ileum. *J. Physiol. (Lond.)* 534: 465–478, 2001.

Stricker EM. Extracellular fluid volume and thrist. *Am J Physiol* 211: 232-238, 1966.

Stricker EM. Some physiological and motivational properties of the hypovolemic stimulus for thirst. *Physiol Behav* 3: 379-385, 1968.

Stricker EM. The renin-angiotensin system and thirst: A reevaluation. II. Drinking elicited by caval ligation or isoproterenol. *J Comp Physiol Psychol* 91: 1220-1231, 1977.

Stricker EM. Thirst and sodium appetite after colloid treatment in rats. *J Comp Physiol Psychol* 95: 1-25, 1981.

Stricker EM. Thirst and sodium appetite after colloid treatment in rats: role of the renin-angiotensin-aldosterone system. *Behav Neurosci* 97: 725-737, 1983.

Stricker EM, Bushey MA, Hoffmann ML, McGhee M, Cason AM, and Smith JC. Inhibition of NaCl appetite when DOCA-treated rats drink saline. *Am J Physiol Regul Integr Comp Physiol* 292: R652-R662, 2007.

Stricker EM, Callahan JB, Huang W, and Sved AF. Early osmoregulatory stimulation of neurohypophyseal hormone secretion and thirst after gastric NaCl loads. *Am J Physiol Regulatory Integrative Comp Physiol* 282: R1710-R1717, 2002.

Stricker EM, Curtis KS, Peacock K, and Smith JC. Rats with area postrema lesions have lengthy eating and drinking bouts when fed ad libitum: Implications for feedback inhibition of ingestive behavior. *Behav Neurosci* 111: 624-633, 1997.

Stricker EM, Gannon KS, and Smith JC. Thirst and salt appetite induced by hypovolemia in rats: Analysis of drinking behavior. *Physiol Behav* 51: 27-37, 1992

Stricker EM and Hoffmann ML. Inhibition of vasopressin secretion when dehydrated rats drink water. *Am J Physiol Regulatory Integrative Comp Physiol* 289: R1238-R1243, 2005.

Stricker EM and Jalowiec JE. Restoration of intravascular fluid volume following acute hypovolemia in rats. *Am J Physiol* 218: 191-196, 1970.

Stricker EM, Vagnucci AH, McDonald RH Jr, and Leenen FH. Renin and aldosterone secretions during hypovolemia in rats: relation to NaCl intake. *Am J Physiol Regulatory Integrative Comp Physiol* 237: R45-51, 1979.

Stricker EM and Verbalis JG. Interaction of osmotic and volume stimuli in regulation of neurohypophyseal secretion in rats. *Am J Physiol Regulatory Integrative Comp Physiol* 250: R267-R275, 1986.

Stricker EM and Verbalis JG. Central inhibitory control of sodium appetite in rats: correlation with pituitary oxytocin secretion. *Behav Neurosci* 101: 560-567, 1987.

Stricker EM and Verbalis JG. Central inhibition of salt appetite by oxytocin in rats. *Reg Peptides* 66: 83-85, 1996.

Tordoff MG, Schulkin J, and Friedman MI. Hepatic contribution to satiation of salt appetite in rats. *Am J Physiol Regulatory Integrative Comp Physiol* 251: R1095-R1102, 1986.

Tordoff MG, Schulkin J, and Friedman MI. Further evidence for hepatic control of salt intake in rats. *Am J Physiol Regulatory Integrative Comp Physiol* 253: R444-R449, 1987.

Thrasher TN, Nistal-Herrera JF, Keil LC, and Ramsay DJ. Satiety and inhibition of vasopressin secretion after drinking in dehydrated dogs. *Am J Physiol Endocr Metab* 240: E394-E401, 1981.

Waldbillig RJ and Lynch WC. Oroesophageal factors in the patterning of drinking. *Physiol Behav* 22: 205–209, 1979.